

## Claims

1. A conjugate comprising a dye labeled nucleobase of the form:

(1) B-L-D, wherein B is a nucleobase, L is an anionic linker, and D comprises at least one fluorescent dye, or

(2) B-L1-D1-L2-D2, wherein B is a nucleobase, L1 and L2 are linkers such that at least one of L1 and L2 is an anionic linker, and D1 and D2 are members of a fluorescent donor/acceptor pair, such that one of D1 and D2 is a donor dye capable of absorbing light at a first wavelength and emitting energy in response thereto, and the other of D1 and D2 is an acceptor dye capable of absorbing energy emitted by the donor dye and fluorescing at a second wavelength in response thereto.

2. The conjugate of claim 1 wherein the dye-labeled nucleobase is of the form B-L-D.

3. The conjugate of claim 2 wherein L comprises a sulfonic acid moiety.

4. The conjugate of claim 2 wherein L comprises a sulfonated benzene moiety.

5. The conjugate of claim 2 wherein L comprises an anionic phosphate moiety.

6. The conjugate of claim 5 wherein the anionic phosphate moiety is a phosphate diester moiety, and the phosphorus atom of the phosphate diester moiety is located in L within a chain of linker atoms that connect B to D.

7. The conjugate of claim 2 wherein L comprises an anionic phosphonate moiety.

8. The conjugate of claim 7 wherein the phosphonate moiety is a phosphonate monoester moiety, and the phosphorus atom of the phosphonate monoester moiety is located in L within a chain of linker atoms that connect B to D.

9. The conjugate of claim 2 wherein L comprises a carboxylic acid moiety.

10. The conjugate of claim 9 wherein the carboxylic acid moiety is a carboxyl benzene moiety.

11. The conjugate of claim 2 wherein L comprises 4 to 20 chain atoms.

5 12. The conjugate of claim 2 wherein D comprises at least one xanthene, rhodamine, dibenzorhodamine, fluorescein, [8,9]benzophenoxazine, cyanine, phthalocyanine, squaraine, or bodipy dye.

13. The conjugate of claim 12 wherein D comprises at least one fluorescein or rhodamine.

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14. The conjugate of claim 1 wherein B comprises adenine, 7-deazaadenine, 7-deaza-8-azaadenine, cytosine, guanine, 7-deazaguanine, 7-deaza-8-azaguanine, thymine, uracil, or inosine.

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15. The conjugate of claim 1 wherein the labeled nucleobase is of the form B-L1-D1-L2-D2.

16. The conjugate of claim 15 wherein L1 is an anionic linker.

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17. The conjugate of claim 16 wherein L1 comprises a sulfonic acid moiety.

18. The conjugate of claim 16 wherein L1 comprises a sulfonated benzene moiety.

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19. The conjugate of claim 16 wherein L1 comprises an anionic phosphate moiety.

20. The conjugate of claim 19 wherein the anionic phosphate moiety is a phosphate diester moiety, and the phosphorus atom of the phosphate diester moiety is located in L1 within a chain of linker atoms that connect B to D1.

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21. The conjugate of claim 16 wherein L1 comprises an anionic phosphonate moiety.

22. The conjugate of claim 21 wherein the phosphonate moiety is a phosphonate monoester moiety, and the phosphorus atom of the phosphonate monoester moiety is located in L1 within a chain of linker atoms that connect B to D1.

23. The conjugate of claim 16 wherein L1 comprises a carboxylic acid moiety.

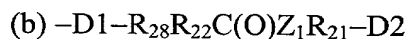
24. The conjugate of claim 23 wherein the carboxylic acid moiety is a carboxy benzene moiety.

25. The conjugate of claim 15 wherein L1 comprises 4 to 20 linker chain atoms.

26. The conjugate of claim 15, wherein at least one of D1 or D2 comprises a xanthene, rhodamine, dibenzorhodamine, fluorescein, [8,9]benzophenoxazine, cyanine, phthalocyanine, or squaraine dye.

27. The conjugate of claim 15 wherein L2 is not an anionic linker.

28. The conjugate of claim 27 wherein D1-L2-D2 comprises structure (a), (b) or (c) below:



wherein:

R<sub>21</sub> is C<sub>1</sub>-C<sub>5</sub> alkylidyl,

Z<sub>1</sub> is NH, S, or O,

R<sub>22</sub> is ethenediyl, ethynediyl, 1,3-dienediyl, diynediyl, 1,3-diynediyl, a 5- or 6-membered ring having at least one unsaturated bond or a fused ring structure, and

R<sub>28</sub> is a bond or spacer group.

29. The conjugate of claim 28, wherein D1 is a donor dye and D2 is an acceptor dye.

30. The conjugate of claim 28 wherein R<sub>22</sub> is a five or six membered ring selected from the group consisting of cyclopentene, cyclohexene, cyclopentadiene, cyclohexadiene, furan, thiofuran, pyrrole, isopyrrole, isoazole, pyrazole, isoimidazole, pyran, pyrone, benzene, pyridine, pyridazine, pyrimidine, pyrazine oxazine, indene, benzofuran, thionaphthene, indole and naphthalene.

31. The conjugate of claim 28 wherein  $R_{28}$  is of the form  $-R_{29}Z_2-C(O)-$ , wherein  $R_{29}$  is  $C_1-C_5$  alkylidyl, and  $Z_2$  is NH, S, or O.

5 32. The conjugate of claim 28 wherein  $R_{21}$  is  $CH_2$ ,  $Z_1$  is NH,  $R_{22}$  is phena-1,4-diyl, and  $R_{29}$  is  $CH_2$ , and  $Z_2$  is NH.

33. The conjugate of claim 27 wherein L2 comprises up to 20 linker chain atoms.

10 34. The conjugate of claim 27 wherein D1 is a donor dye and D2 is an acceptor dye.

35. The conjugate of claim 15 wherein L2 is an anionic linker.

36. The conjugate of claim 35 wherein L2 comprises a sulfonic acid moiety.

37. The conjugate of claim 35 wherein L2 comprises a sulfonated benzene moiety.

38. The conjugate of claim 35 wherein L2 comprises an anionic phosphate moiety.

15 20 39. The conjugate of claim 38 wherein the anionic phosphate moiety is a phosphate diester moiety, and the phosphorus atom of the phosphate diester moiety is located in L2 within a chain of linker atoms that connect D1 to D2.

25 40. The conjugate of claim 35 wherein L2 comprises an anionic phosphonate moiety.

41. The conjugate of claim 40 wherein the phosphonate moiety is a phosphonate monoester moiety, and the phosphorus atom of the phosphonate monoester moiety is located in L2 within a chain of linker atoms that connect D1 to D2.

30 42. The conjugate of claim 35 wherein L2 comprises a carboxylic acid moiety.

43. The conjugate of claim 42 wherein the carboxylic acid moiety is a carboxy benzene moiety.

44. The conjugate of claim 35 wherein L2 comprises up to 20 linker chain atoms.

45. The conjugate of claim 35 wherein D1 is a donor dye and D2 is an acceptor dye.

46. The conjugate of claim 15 wherein L2 is an anionic linker and L1 is not an anionic linker.

47. The conjugate of claim 46 wherein L2 comprises a sulfonic acid moiety.

48. The conjugate of claim 46 wherein L2 comprises a sulfonated benzene moiety.

49. The conjugate of claim 46 wherein L2 comprises an anionic phosphate moiety.

50. The conjugate of claim 49 wherein the anionic phosphate moiety is a phosphate diester moiety, and the phosphorus atom of the phosphate diester moiety is located in L2 within a chain of linker atoms that connect D1 to D2.

51. The conjugate of claim 46 wherein L2 comprises an anionic phosphonate moiety.

52. The conjugate of claim 51 wherein the phosphonate moiety is a phosphonate monoester moiety, and the phosphorus atom of the phosphonate monoester moiety is located in L2 within a chain of linker atoms that connect D1 to D2.

53. The conjugate of claim 46 wherein L2 comprises a carboxylic acid moiety.

54. The conjugate of claim 53 wherein the carboxylic acid moiety is a carboxy benzene moiety.

55. The conjugate of claim 46 wherein L2 comprises up to 20 linker chain atoms.

56. The conjugate of claim 46, wherein at least one of D1 or D2 comprises a xanthene, rhodamine, dibenzorhodamine, fluorescein, [8,9]benzophenoxazine, cyanine, phthalocyanine, or squaraine dye.

57. The conjugate of claim 46 wherein D1 is a donor dye and D2 is an acceptor dye.

58. The conjugate of claim 46 wherein L1 comprises one of the following moieties:

- 5     $-\text{C}\equiv\text{CCH}_2\text{NH}-$ ,  $-\text{C}\equiv\text{CCH}_2\text{NHC}(\text{O})(\text{CH}_2)_5\text{NH}-$ ,  $-\text{C}=\text{CC}(\text{O})\text{NH}(\text{CH}_2)_5\text{NH}-$ ,  
     $-\text{C}\equiv\text{CCH}_2\text{OCH}_2\text{CH}_2\text{NH}-$ ,  $-\text{C}\equiv\text{CCH}_2\text{OCH}_2\text{CH}_2\text{OCH}_2\text{CH}_2\text{NH}-$ ,  $-\text{C}\equiv\text{C}-\text{CH}_2\text{OCH}_2\text{CH}_2-\text{NH}-$ , and  
     $-\text{C}=\text{C}(p\text{-C}_6\text{H}_5)\text{OCH}_2\text{CH}_2\text{NH}-$ .

59. A labeled nucleoside triphosphate comprising a conjugate of claim 1.

60. The labeled nucleoside triphosphate of claim 59 which is not 3'-extendable.

61. The labeled nucleoside triphosphate of claim 60 which is a 2',3'-dideoxynucleotide or 3'-fluoro-2',3'-dideoxynucleotide.

62. The labeled nucleoside triphosphate of claim 59 which contains a 3'-hydroxyl group.

63. A polynucleotide comprising a conjugate of claim 1.

64. The polynucleotide of claim 63 wherein the conjugate is located on a 3' terminal nucleotide subunit.

65. The polynucleotide of claim 64 wherein the 3' terminal nucleotide subunit is not extendable.

66. The polynucleotide of claim 65 wherein the 3' terminal nucleotide subunit is a 2',3'-dideoxynucleotide or 3'-fluoro-2',3'-dideoxynucleotide.

67. The polynucleotide of claim 64 which contains a 3'-hydroxyl group.

68. The polynucleotide of claim 63 wherein the conjugate is located on a non-terminal nucleotide subunit.

69. A mixture comprising a plurality of different-sequence polynucleotides, wherein at least one polynucleotide contains a conjugate of claim 1.

70. The mixture of claim 69 which comprises at least two different-sequence polynucleotides that each contain a conjugate of claim 1, such that each conjugate attached to the different-sequence polynucleotides may be the same or different.

71. The mixture of claim 69 which comprises four classes of polynucleotides, wherein the polynucleotides in each class terminate with a distinct terminator subunit type that contains a distinct conjugate of claim 1 to identify the polynucleotides in that class.

72. A nucleic acid sequencing kit comprising at least one labeled nucleoside triphosphate of claim 54 and one or more of the following components:

a 3'-extendable primer,

a polymerase enzyme,

one or more 3' extendable nucleotides which are not labeled with conjugate, and/or

a buffering agent.

73. The kit of claim 72 wherein at least one labeled nucleoside triphosphate is nonextendable.

74. The kit of claim 72 which comprises four different labeled nucleoside triphosphates which are complementary to A, C, T and G, and each of which contains a distinct conjugate of claim 1.

75. The kit of claim 74 wherein the four different labeled nucleoside triphosphates are nonextendable.

76. The kit of claim 74 wherein the four different labeled nucleoside triphosphates are extendable ribonucleoside triphosphates.

77. The kit of claim 72 wherein the conjugate has the form B-L1-D1-L2-D2.

78. The kit of claim 77 wherein the donor dyes in the four different labeled nucleoside triphosphates are the same.

79. The kit of claim 78 wherein the donor dye is an orthocarboxyfluorescein.

80. The kit of claim 78 wherein the donor dye is a 4,7-dichloro-orthocarboxyfluorescein.

81. A method of forming a labeled polynucleotide strand, the method comprising reacting together a duplex polynucleotide comprising (i) a 3'-extendable strand which is hybridized to a complementary template strand having a 5' overhang, (ii) a template-dependent polymerase enzyme and (iii) a labeled nucleoside triphosphate containing a conjugate of claim 1, under conditions effective to form a labeled polynucleotide containing the conjugate.

82. A method of sequencing a target polynucleotide sequence, the method comprising forming four classes of polynucleotides which are complementary to a target polynucleotide sequence, by template-dependent primer extension, wherein the polynucleotides in each class terminate with a different terminator subunit type that contains a distinct conjugate of claim 1 to identify the polynucleotides in that class, and

separating the polynucleotides of the four classes on the basis of size to obtain a mobility pattern, and determining the sequence of the target polynucleotide sequence from the mobility pattern.

83. The method of claim 82 wherein the terminator subunits are nonextendable.

84. The method of claim 82 wherein the terminator subunits contain a 3'-hydroxyl group.

85. A method of identifying one or more polynucleotide(s), the method comprising forming one or more labeled different-sequence polynucleotides, wherein each different-sequence polynucleotide contains a unique conjugate of claim 1, and

separating the one or more labeled different-sequence polynucleotides by electrophoresis so as to separate different-sequence polynucleotides on the basis of size, and

identifying each different-sequence polynucleotide on the basis of its electrophoretic mobility and fluorescence signal.